Genetic structure and heterozygosity-related fitness effects in the marine snail *Littorina littorea*

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Abstract. The relationships among rate of oxygen consumption under routine and starved conditions, soft tissue dry weight, shell growth rate and heterozygosity at polymorphic allozyme loci were investigated in 86 individuals of the common periwinkle, *Littorina littorea* (Linnaeus, 1758). Oxygen consumption was measured at the time of collection and after 14 days starvation using a micro-Winkler method. Soft tissue dry weight was estimated from shell height using a regression equation developed from analysis of a set of 34 additional snails. Shell growth rate was estimated as distance between successive lines in thin sections through the growing edge of the shell. Heterozygosity was studied using conventional starch gel electrophoretic techniques to examine enzyme systems controlled by 34 loci; 11 of the loci were polymorphic. The mean observed heterozygosity per locus per individual was 0.073. Continuous variables were adjusted for differences in estimated dry weight by using linear regression to convert all measurements to a standard dry weight. Only for growth rate was there a significant positive association with dry weight. The weight-adjusted values were then tested for an effect of heterozygosity using linear regression. There was no evidence for an association between level of heterozygosity and oxygen consumption under either routine or starved conditions. However, starvation caused a significant depression in oxygen consumption, in paired comparisons. There was no detectable association between heterozygosity and shell growth rate.

Genetic studies of marine mollusc populations have reported several unexpected findings, including deficiencies in the numbers of heterozygous individuals and correlations between estimates of an individual's overall level of heterozygosity and various surrogate measures of fitness such as growth or viability (Mitton and Grant, 1984; Zouros and Foltz, 1984, 1987). Among marine molluscs, there is abundant evidence for age- or size-dependent changes in allele frequencies and in the level of observed heterozygosity for numerous allozyme loci. One common pattern is for the heterozygosity to increase (or the deficiency of heterozygotes to decrease) among older or larger animals, suggesting an apparent viability advantage of more heterozygous individuals. There is also an extensive literature reporting positive correlations between allozyme heterozygosity and growth rate in marine molluscs and other organisms; several conclusions have emerged from this work. First, the apparent correlation between heterozygosity and growth rate can be related at the physiological level to either a lower cost of routine metabolism (Koehn and Shumway, 1982; Garton, 1984), a higher feeding rate (Garton, 1984; Holley and Foltz, 1987), or both, in highly heterozygous individuals. Hawkins *et al.* (1986, 1989) have attempted to integrate these findings with more traditional concepts in molluscan physiology. They argue that the greater metabolic efficiency of more heterozygous individuals results from lower rates of protein turnover in such individuals, not from lower rates of energy metabolism. Further, they suggest that low intensities of protein turnover reduce the energy required for maintenance, with some of the energy saved being used to support increased feeding rates. A second approach has been to compare heterozygosity-fitness correlations from various studies and organisms in a search for general trends. For example, Zouros (1987) reported that significant positive correlations between heterozygosity and fitness components were more likely to be found in marine molluscs if (1) the allozyme loci showed deficiencies in the numbers of heterozygous individuals (compared to Hardy-Weinberg expectations), and (2) the sample came from a natural population rather than laboratory populations derived from a small number of parents. These two findings suggest an influence of population genetic structure on the occurrence of heterozygosity-fitness correlations. The association between degree of heterzygote deficiency and occurrence of heterozygosity-fitness correlations has not been satisfactorily explained, and seems counter-intuitive. Although several different hypotheses have been proposed to explain this association (see below), present data are insufficient to decide among them. More data

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are needed, particularly to determine if the association between heterozygote deficiency and heterozygosity/fitness correlations is of wide occurrence. The present study examines genetic structure, heterozygosity, weight, growth rate and oxygen consumption rate in a natural population sample of the marine snail *Littorina littorea* (Linnaeus, 1758).

MATERIALS AND METHODS

A total of 86 *Littorina littorea* (shell height range 16.6—26.2 mm) was collected near West Boothbay Harbor, Maine (during the same tidal cycle and from the same shore height) in the spring of 1987. Immediately after collection, each snail was marked individually with a small plastic tag and its (routine) oxygen consumption rate determined. The snails were then held in tanks supplied with running seawater without food for 14 days, after which rates of oxygen consumption were again measured. Seawater temperatures ranged from 8-12°C during the experiments, and all measurements of oxygen consumption were made under ambient conditions. Oxygen consumption rates were measured on individual animals in 70 ml experimental chambers using a micro-Winkler technique (Burke, 1962). The snails were allowed to equilibrate for 1 hr, during which time the water was aerated. Measurements were carried out over a period of approximately 3 hrs; total oxygen concentration in the experimental chambers never fell below 70% saturation. Controls were run using identical vessels with no animals. The snails were then placed in large (2.0 m x 0.5 m x 0.5 m) cages at mid-tide level for four weeks. Ample supplies of *Ulva* and *Enteromorpha* were maintained in the cages as a food source. The snails were removed from the cage and the shell height of each animal was measured. The free growing edge of each shell was removed with a diamond saw, and the soft tissues were removed and frozen at -80°C for later allozyme analysis.

Shells were preserved in 70% alcohol containing a small quantity of borax and air-shipped to Menai Bridge, Wales, for growth measurements in the laboratory of D. J. Crisp. The most recently deposited 2 or 3 mm at the lip of each shell were embedded in resin. Sections were cut parallel to the direction of growth, and the cut surface ground on wet and dry paper and polished on a cloth soaked in "Brasso." The polished surface was etched in 0.01 M HCl for 10-20 min, and acetate peel replicas of the dry surface prepared using the technique described by Ekaratne and Crisp (1982). The peels were examined microscopically and the shell growth rate for each animal estimated from the width of the most recently deposited band. The growth bands were reasonably assumed to correspond to the most recent tidal cycle, because similar bands have been shown to be tidally-produced in *Littorina littorea* from Menai Strait, Wales (Ekaratne and Crisp, 1982, 1984). Soft tissue dry weight of each animal was estimated by a regression equation obtained from a set of 34 additional snails collected near West Boothbay Harbor. This set had a mean shell height of 18.6 mm (range 6.5—28.2 mm) and a mean soft tissue dry weight of 0.146 g (range 0.002—0.384 g). The regression equation relating soft tissue dry weight (Y) and shell height (X) was $\log_e(Y) = -12.44 + 3.49 \cdot \log_e(X)$, $P < 0.0001$, $R^2 = 0.98$. The mean estimated soft tissue dry weight of the 86 snails was 0.153 g (range 0.074—0.314 g).

The frozen tissue samples were air-shipped to the laboratory of D. W. Foltz, where they were processed and analyzed electrophoretically by standard methods (e.g. Harris and Hopkinson, 1976). In all, 34 enzyme loci were resolved. Alleles at each polymorphic locus were designated by numbers indicating mobility relative to the most common allele at that locus, which was designated "100"; negative numbers indicated cathodally migrating bands. For enzymes with multiple isozymic forms, loci were numbered sequentially starting with the most anodally-migrating system. Allele frequencies, fixation indices (F) and observed and expected heterozygosities for each locus were calculated using standard formulas (e.g. Hedrick, 1983). All data were analyzed by the Statistical Analysis System (SAS Institute, Inc., 1985).

RESULTS

Of the 34 enzyme loci examined, 11 were polymorphic (see Table 1 for allele frequencies and single-locus observed heterozygosities). The fixation index (F) for each polymorphic locus was close to 0, indicating no significant departures from Hardy-Weinberg equilibrium. The average F was 0.003 (range -0.051—0.131). The average number of heterozygous loci per individual was 2.49 (range 0—5). The frequency of each heterozygosity class was plotted and compared with the expected frequency derived from the 11 single-locus expected heterozygosities (Fig. 1). The distribution of observed heterozygosity was more platykurtic than the expected distribution, with an excess of both low-heterozygosity and high-heterozygosity individuals.

Continuous variables (growth rate, routine and starved oxygen consumption rate) were $\log_e$-transformed prior to analysis (to help ensure that each dependent variable had a normal distribution and uniform variance) and were adjusted for differences in estimated soft tissue dry weight by using linear regression to convert all measurements to a standard dry weight (0.146 grams). As expected, there was a significant positive effect of dry weight on growth rate ($P < 0.03$), but no detectable effect of dry weight on routine ($P > 0.07$) or starved ($P > 0.26$) VO_2. The finding of no association between oxygen consumption under routine or starved conditions and estimated soft tissue dry weight was unexpected. It differs from previous studies of *Littorina littorea* (e.g. Newell and Roy, 1973) that have found a positive correlation between oxygen consumption and dry tissue weight. The lack

of a correlation is most likely due to the absence of extremely low-weight individuals in the present sample. For example, in Newell and Roy's (1973) study the range for estimated soft tissue dry weight was 2—160 mg, whereas the corresponding range in the present study was 70—300 mg. There was a significant depression in oxygen consumption after starvation for 14 days. Previous studies of the effect of starvation on oxygen consumption in pulmonate and prosobranch molluscs (summarized by Studier and Pace, 1978) have variously found that oxygen consumption rates increase, decrease or remain constant in starved animals. Factors responsible for such different responses could include length of starvation period, acclimation temperature or sex differences. Fig. 2 presents growth rate (μm/tidal cycle), adjusted for dry weight differences, and dry weight (in grams) for different levels of heterozygosity. Multiple-locus heterozygosity was not a significant source of variation for either adjusted growth rate ($P>0.07$, $R^2=0.04$) or dry weight ($P<0.77$), $R^2<0.01$). Single-locus heterozygosity for each of the 11 polymorphic loci also was not a significant source of variation for either adjusted growth rate or dry weight, after allowing for multiple tests (results not shown). Fig. 3 presents Vo_2

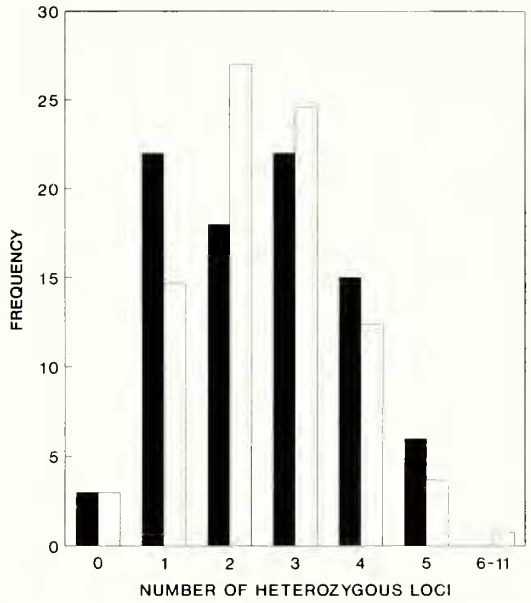

Fig. 1. Observed (filled bars) and expected (open bars) numbers of individuals with various numbers of heterozygous loci (out of 34 examined loci) in *Littorina littorea*.

Table 1. Enzyme commission (E.C.) numbers, allele frequencies and observed heterozygosities (H_0), ± 1 standard error, at 11 polymorphic allozyme loci in *Littorina littorea*.

Locus	E.C. No.	Allele	Frequency	Locus	E.C. No.	Allele	Frequency
Acon-1	4.2.1.3	107	.023 ± .011	*Pep*	3.4.-.-	112	.017 ± .010
		100	.971 ± .013			100	.983 ± .010
		95	.006 ± .006			H_0	.035 ± .020
		H_0	.058 ± .025				
Acon-2	4.2.1.3	208	.006 ± .006	*6-Pgd*	1.1.1.44	175	.029 ± .013
		146	.122 ± .025			147	.238 ± .032
		100	.872 ± .025			133	.047 ± .016
		H_0	.232 ± .046			100	.686 ± .035
						H_0	.488 ± .054
Ck	2.7.3.2	127	.052 ± .017	*Pgi*	5.3.1.9	107	.047 ± .016
		119	.012 ± .008			100	.936 ± .019
		100	.936 ± .019			93	.011 ± .008
		H_0	.128 ± .036			85	.006 ± .006
						H_0	.128 ± .036
Est-1	3.1.1.1	105	.011 ± .008	*Pgm-1*	5.4.2.2	100	.913 ± .022
		100	.989 ± .008			71	.087 ± .022
		H_0	.023 ± .016			H_0	.151 ± .038
Est-3	3.1.1.1	-119	.273 ± .034	*Pgm-2*	5.4.2.2	126	.047 ± .016
		-100	.401 ± .038			111	.106 ± .023
		-67	.303 ± .035			100	.694 ± .035
		-59	.023 ± .011			83	.153 ± .028
		H_0	.698 ± .050			H_0	.447 ± .054
Glydh	1.1.1.29	116	.064 ± .019				
		100	.936 ± .019	Average*		H_0	.073 ± .005
		H_0	.105 ± .033				

*Average H_0 includes the following 23 monomorphic loci: *Ada* (3.4.4.4); *Adh* (1.5.1.17); *Ald* (4.1.2.13); *Alkp* (3.1.3.1); *Ark* (2.7.3.3); *Est-2* (3.1.1.1); *Fum* (4.2.1.2); *Got* (2.6.1.1); *G-6-Pdh* (1.1.1.49); *Gpt* (2.6.1.2); *Gpdh* (1.1.1.8); *Idh-1* (1.1.1.42); *Idh-2* (1.1.1.42); *Ipo* (1.15.1.1); *Lap* (3.4.-.-); *Mdh* (1.1.1.37); *Me* (1.1.1.40); *Mpi* (5.3.1.8); *Nsp* (2.4.2.1); *Odh* (1.5.1.11); *Pep (LGG)* (3.4.-.-); *Sdh* (1.1.1.14); *Xdh* (1.1.1.204).

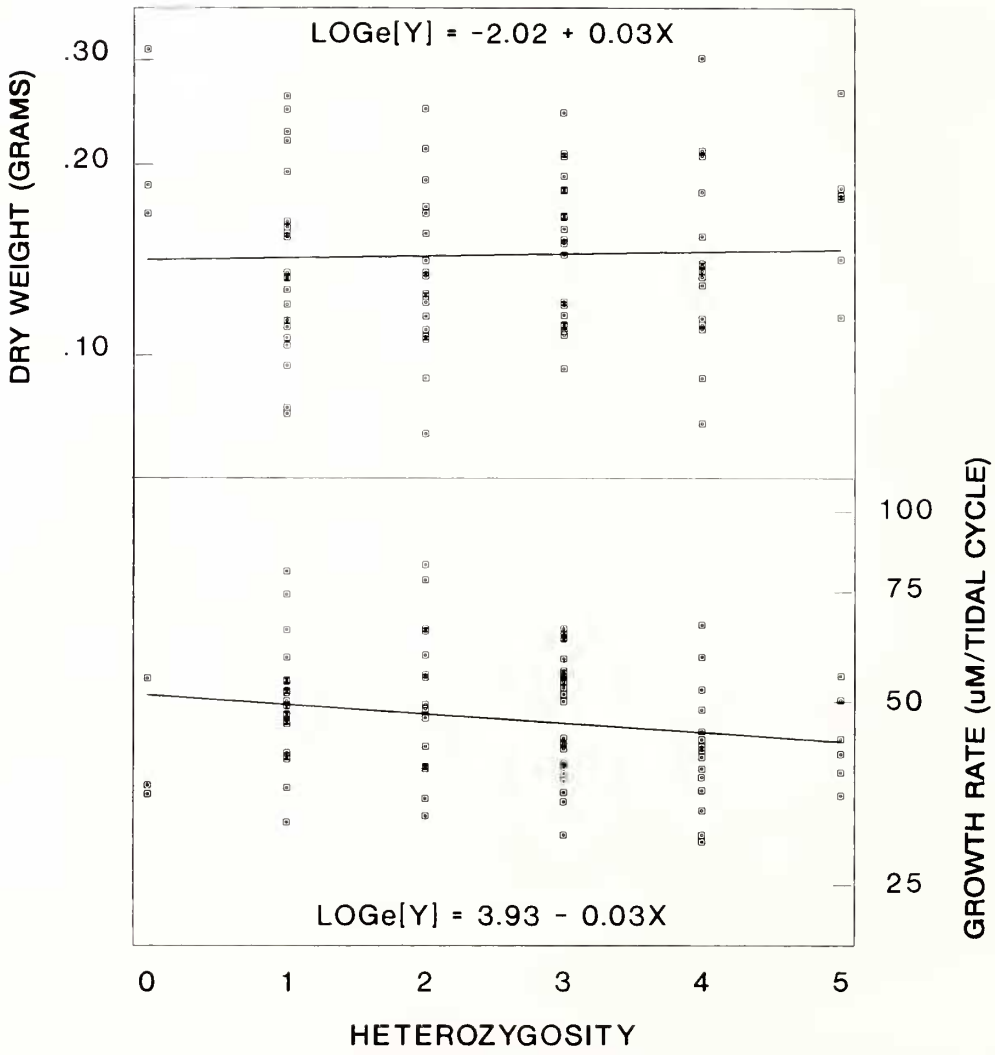

Fig. 2. Semi-logarithmic plots of estimated soft tissue dry weight (upper panel) and growth rate (lower panel) verus heterozygosity in *Littorina littorea.* Regression lines and equations are also shown, but are not statistically significant.

(ml $O_2 \cdot hr^{-1}$) under routine and starved conditions for different levels of heterozygosity. The effect of heterozygosity on adjusted oxygen consumption rate was not significant when tested by linear regression, under either routine ($P > 0.65$) or starved ($P > 0.85$) conditions. There was a significant reduction ($P < 0.0001$ by paired t-test) in adjusted oxygen consumption rate under starved conditions (mean = 0.092 ml $O_2 \cdot hr^{-1}$) when compared to the corresponding mean value (0.113) under initial conditions.

DISCUSSION

The observed heterozygosity in this study (0.073 ± 0.005) is slightly higher than that reported (0.04) in previous studies of *Littorina littorea* (Fevolden and Garner, 1987; Janson, 1987). Fevolden and Garner (1987) found no evidence for heterozygote excesses or deficiencies. They also looked for genetic differences between fast-growing and slow-growing snails at the *6-Pgd* locus, with negative results. The present study extends that conclusion to a larger set of heterozygous loci and to oxygen consumption rate comparisons. Plots of VO_2 versus heterozygosity for both routine and starved conditions gave no suggestion of an association for either treatment, although the three snails with 0 observed heterozygosity had very low oxygen consumption rates. As seen in Fig. 2, these animals also had very high weights. Although the oxygen consumption rates were adjusted for the (linear) effect of $\log_e$-transformed dry weight, there could have been some residual effect of weight differences on oxygen consumption.

The previous literature on allozyme heterozygosity-fitness correlations has been summarized by Mitton and Grant (1984), Zouros and Foltz (1987) and Zouros (1987). As noted

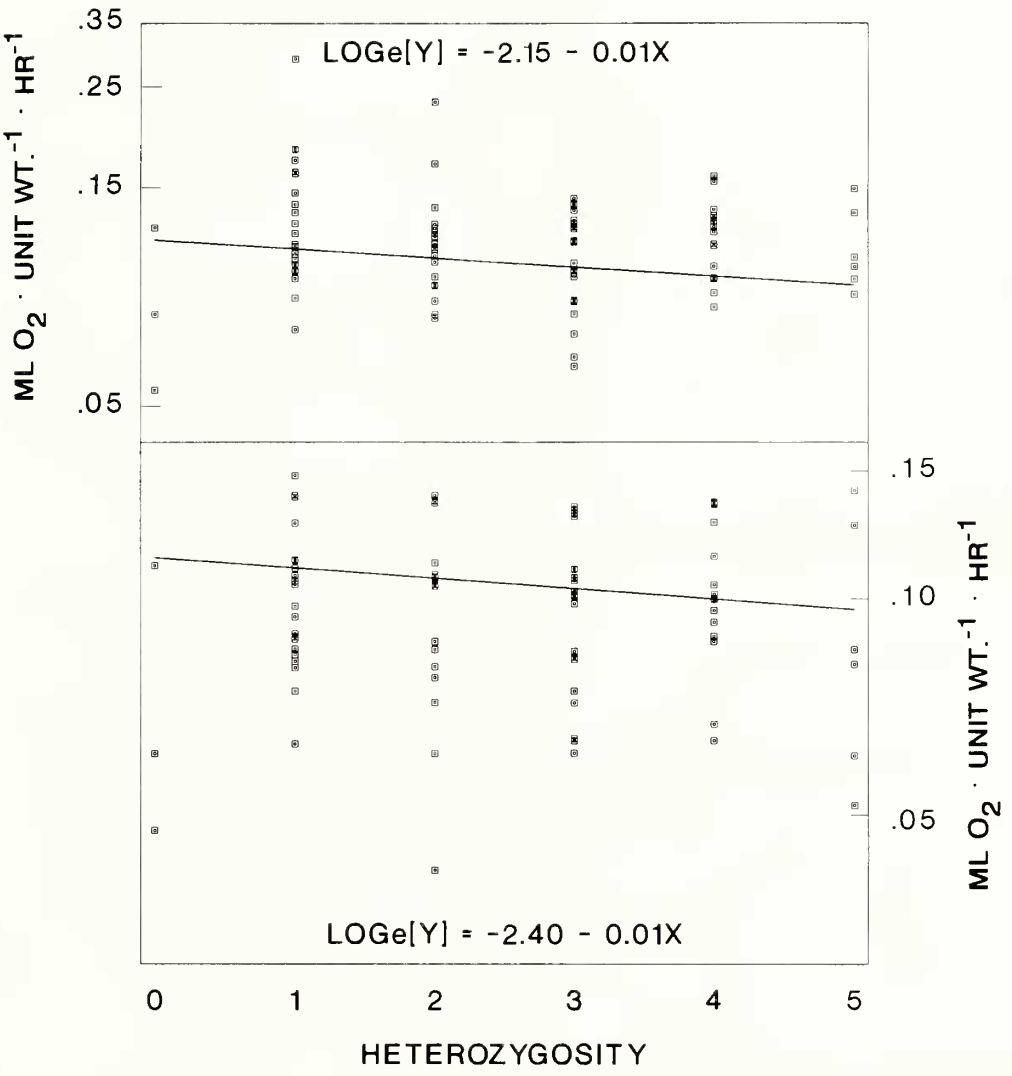

Fig. 3. Semi-logarithmic plots of oxygen consumption rate under fed conditions (upper panel) and under 14-day starvation conditions (lower panel) versus heterozygosity in *Littorina littorea*. Regression lines and equations are also shown, but are not statistically significant.

above, heterozygosity-fitness correlations are more likely to be found when the same set of allozyme loci exhibits a deficiency in the number of heterozygous individuals than when they do not. The data obtained for *Littorina littorea* in this study and by Fevolden and Garner (1987) are consistent with the suggestion that heterozygosity-fitness correlations in natural populations of marine molluscs are largely absent when genotype frequencies closely approximate the Hardy-Weinberg expectations. Two previous studies of heterozygosity and growth rate in marine gastropods (Fujino, 1978; Garton, 1984) reported the co-occurrence of heterozygote deficiencies and heterozygosity-size (or heterozygosity-growth rate) correlations. Despite the seeming contradiction between deficiencies in the numbers of heterozygotes (compared to Hardy-Weinberg expectations) and apparent heterozygote superiority at the same loci, positive associations between these two phenomena have been found in numerous studies. This pattern occurs when different species are compared (Zouros, 1987; Zouros and Mallet, 1989) and also when different loci within a single species are compared (Gaffney *et al.*, 1990). As reviewed by Zouros *et al.* (1988) and Gaffney *et al.* (1990), at least three explanations can potentially explain the co-occurrence of heterozygote deficiencies and heterozygosity-fitness correlations at the same set of loci. First, molecular imprinting could account for both phenomena (Chakraborty, 1989). Second, a high rate of chromosomal mutation (Thiriot-Quievreux, 1986; Thiriot-Quievreux *et al.*, 1988) and/or a high rate of single-gene mutation, could be responsible for apparent heterozygote deficiencies through production of null mutations, where nulls

could either be point mutations resulting in loss of activity at a single allozyme locus or else multi-locus deletions with presumably larger fitness effects. A high mutation rate could also cause apparent heterozygosity-fitness correlations, either through direct reduction in fitness of allozyme active/null heterozygous genotypes (Zouros and Foltz, 1987) or through associative overdominance (Zouros, 1987). Third, partial inbreeding will generate both a deficiency of heterozygotes at individual loci and an inter-locus correlation in the degree of heterozygosity (Haldane, 1949; Bennett and Binet, 1956). These inter-locus correlations can generate apparent heterozygote superiority through inbreeding depression (Strauss, 1986; Bush *et al.*, 1987) even in the absence of any direct fitness effect of the loci examined. As yet, there is insufficient knowledge about the genetics and population structure of marine molluscs to allow these possibilities to be tested.

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